REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2002	Annual (1 Jun 01 - 31 May 02)

4. TITLE AND SUBTITLE
Novel Vectors for Dendritic Cell Transduction

5. FUNDING NUMBERS
DAMD17-00-1-0122

6. AUTHOR(S)
Theresa V. Strong, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Alabama at Birmingham
Birmingham, Alabama 35294-0111

E*Mail: theresa.strong@ccc.uab.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
The development of vaccine approaches for breast cancer has the potential to provide an adjuvant therapy with low toxicity for patients at risk for disease recurrence. We are investigating novel vaccine strategies for breast cancer. The target tumor antigen is carcinoembryonic antigen (CEA), which is highly expressed in most breast tumors. Dendritic cells (DCs) show promise for cancer immunotherapy due to their critical role in mediating immune response. Development of an optimal DC transduction protocol for tumor antigen presentation would represent a significant advancement in DC-based vaccination strategies. We are investigating methods of DC transduction and antigen modification that will elicit the most potent anti-tumor immune response. Tumor challenge and tumor therapy experiments are performed using a syngeneic adenocarcinoma cell line which expresses human CEA (MC38-CEA-2). Because the ability to break tolerance to self antigens is critical for the success of this cancer immunotherapy approach, a CEA-transgenic mouse model will also be used.

14. SUBJECT TERMS	15. NUMBER OF PAGES
immunotherapy, carcinoembryonic antigen, vaccine	8
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

AD_____

Award Number: DAMD17-00-1-0122

TITLE: Novel Vectors for Dendritic Cell Transduction

PRINCIPAL INVESTIGATOR: Theresa V. Strong, Ph.D.

CONTRACTING ORGANIZATION: The University of Alabama at Birmingham
Birmingham, Alabama 35294-0111

REPORT DATE: June 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

Table of Contents

Novel Vectors for Dendritic Cell Transduction
DAMD17-00-1-0122
Principal Investigator: Theresa V. Strong, Ph.D.
Annual Report June 1, 2001 – May 31, 2002

INTRODUCTION:

Cancer immunotherapy approaches aim to enhance the cellular immune response against tumor antigens. Although cytolytic T cells specific for tumor antigens can be isolated from tumor-bearing individuals, it is clear that immune system fails to produce effective antitumor immunity. In recent years, dendritic cells (DCs) have received much attention as their critical role in the elicitation of immune response has been appreciated. Preclinical studies and initial clinical trials using these cells for tumor antigen presentation have produced some encouraging results, however, gene transfer technology for DCs has not yet been optimized. In this project, we are evaluating two novel vector systems for gene transfer into DC. Naked RNA has been shown to mediate gene transfer into dendritic cells and we hypothesize that the use of replicative RNA will enhance transgene expression and improve tumor antigen presentation. As a second gene transfer system we are studying a targeted adenoviral vector developed by our collegues in the Gene Therapy Center at the University of Alabama at Birimingham (1). This adenoviral vector is specifically targeted to the CD40 molecule present on dendritic cells. The target tumor antigen we are studying is CEA, which is highly expressed on human breast cancer and has several features that make it an attractive target for immunotherapy (2). These include its high level expression in most breast tumors, as well as other epithelial tumors, an its probable role in tumorigensis. As a model system to evaluate these vectors, we will deliver the human CEA gene to murine dendritic cells and evaluate the ability of these vectors to break immunological tolerance, induce a CEA-specific immune response and mediate an effective antitumor immune response. The approved specific aims of this project are:

1. **To evaluate the ability of replicative RNA vectors encoding CEA to transfect dendritic cells ex vivo, and elicit an antitumor immune response in a CEA transgenic mouse model of adenocarcinoma.**

2. **To use a bispecific antibody to produce a CD40-targeted adenovirus encoding CEA, and to evaluate its specificity in transducing dendritic cells and efficacy in inducing an antitumor immune response in a CEA transgenic mouse model.**

BODY:

Transgenic CEA animals: CEA transgenic animals were obtained from the National Cancer Institute and were bred in UAB's Transgenic Breeeding Core Facility. As reported in last year's annual report, we developed an assay for identification of transgenic progeny, since, for technical reasons, experimental animals are bred from heterozygous males. Unfortunately, the transgenic facility at UAB reported difficulties in breeding these animals, and after several months, only ten transgenic females had been produced, which was inadequate for our planned experiments. After discussions with the personnel at the Transgenic facility, we decided to bring the animals to our own facility and oversee breeding and testing. Since that time (4/02), we have successfully established the breeding colony, and are steadily producing transgenic animals, which we believe we be produced in adequate numbers for the planned experiments. Thus, although the breeding problem caused a delay in the studies in the transgenic model, we believe the problem has been overcome and do not foresee problems in the coming year with regard to the production of adequate numbers of transgenic animals.

Modification of the CEA antigen to optimize induction of anti-tumor immune responses:
We produced a vector encoding a shortened version of the CEA protein (pCEA70), as described in the first year annual report. This CEA cDNA is internally deleted to remove the second of three repetitive segments, joining repeats I and III. This construct encodes all of the necessary regions to mediate anti-CEA immunity capable of mediating tumor rejection, and allows additional flexibility in modifying the encoded CEA antigen to optimize immune response. To this end, we evaluated the incorporation of foreign antigenic eptitopes in the CEA cDNA as a means to optimize induction of anti-CEA immune response. Two CD4+ cell epitopes, one derived from tetanus toxoid and one derived from measles virus, were genetically fused to the C-terminus of the CEA molecule, and the constructs were sequenced to verify incorporation of the desired sequence. The ability of the modified CEAs was then compared to unmodified CEA in a mouse model (these animals were not transgenic for human CEA). We immunized animals with plasmid DNAs encoding each of the CEAs. A low dose immunization strategy was chosen, as we felt this would allow us to detect subtle differences in the antitumor immune responses elicited by each of the plasmids. Thus, a single immunization of 10ug/mouse was performed. Twenty-one days following the immunization, the mice were challenged with 300,000 MC38-CEA-2 cells, which express CEA. At the immunization dose described, we expect approximately 50% of animals to develop tumors when immunized with unmodified CEA. As evidenced by ELISA assay (not shown), all animals immunized with CEA-containing constructs developed antibodies specific for CEA, at comparable levels. In addition, there did not appear to be any advantage for the T-helper epitope-containing CEA constructs in protecting the animals from tumor challenge:

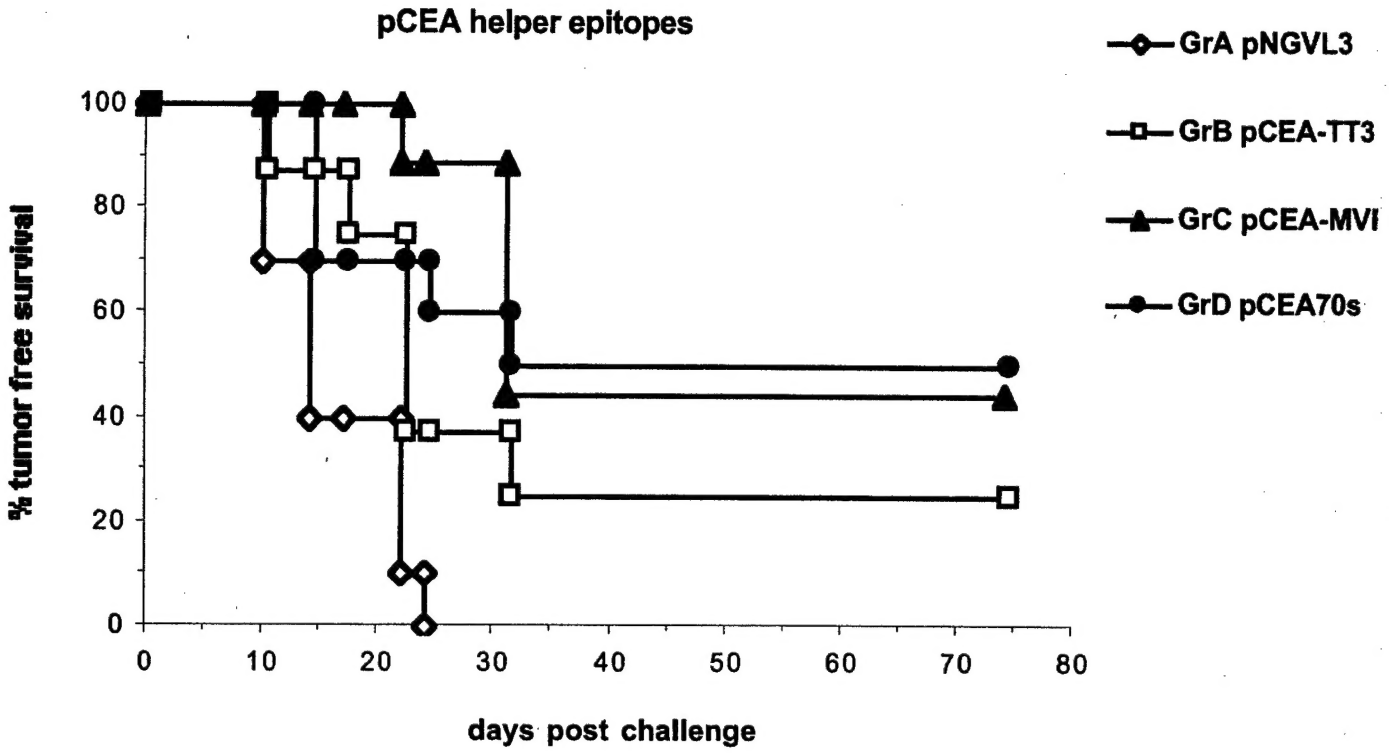

Figure 1. Survival of animals immunized against CEA and challenged with a CEA-expressing adenocarcinoma cell line. Groups of 10 mice were immunized with plasmid DNA encoding a truncated CEA molecule (pCEA70s), truncated CEA with the addition of a tetanus toxoid T-helper epitope (pCEA-TT3), truncated CEA with a measles virus T-helper epitope (CEA-MVF) or empty vector (pNGVL3). Twenty one days after immunization, the mice were challenged with MC38-CEA-2 cells and tumor free survival was monitored.

Thus, addition of T-helper epitopes to the antigen did not result in more potent anti-CEA immune response as determined by these assays.

KEY RESEARCH ACCOMPLISHMENTS

1. Developed a mouse DC preparation protocol for reliable production of mouse DCs (yr1).
2. Produced a truncated CEA antigen, evaluated in a nontransgenic mouse model of adenocarcinoma, demonstrating equivalency with full length CEA (yr1).
3. Evaluated incorporation of T-helper epitopes into CEA (yr2).
4. Established CEA-transgenic breeding colony (yr2).

REPORTABLE OUTCOMES

Abstract presented orally at the Society for Biological Therapy:
Lima, J., C. Jenkins, M. Hamilton, P Triozzi, DR Shaw, TV Strong. A DNA vaccine encoding genetic fusions of CEA and GM-CSF. (copy attached)

CONCLUSIONS

In the second year of this project, we were somewhat limited by difficulties in establishing a productive transgenic breeding colony; however we believe that issue has been addressed and do not anticipate any further difficulties. Our plans for the coming year will be primarily focused on vector development issues, as outlined in our original proposal.

REFERENCES

1. Tillman BW. Hayes TL. DeGruijl TD. Douglas JT. Curiel DT. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based vaccination against human papillomavirus 16-induced tumor cells in a murine model. Cancer Research. 60:5456-63, 2000.

2. Bernistein NL. Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: A review. J Clin Oncol 20:2197-2207, 2002.

A DNA Vaccine Encoding Genetic Fusions of CEA and GMCSF.

Jose Lima, Connie Jenkins, Mary Hamilton, Pierre Triozzi, Denise Shaw, and Theresa Strong.
Department of Medicine and the Comprehensive Cancer Center, The University of Alabama at Birmingham, Birmingham, AL.

Background: Plasmid DNA vaccines encoding tumor antigens have shown promise in animal models, but limited efficacy in the clinical setting. CEA is an intercellular adhesion molecule expressed in human breast, colonic and non-small cell lung cancer and represents a promising tumor-associated antigen for an antitumor immunization. GM-CSF receptors are expressed by mature and immature dendritic cells, and GM-CSF is well established as a potent immune adjuvant, in part due to its ability to recruit and activate dendritic cells.
Objective: Investigate the use of plasmid DNA encoding fusion proteins to better target antigen presenting cells for enhanced immune response.
Methods: As a first step, we constructed two plasmids encoding fusions between carcinoembryonic antigen (CEA) and murine GM-CSF (mGM-CSF). CEA was fused with GM-CSF in the carboxy or amino terminal, with a short, flexible linker joining the two moieties. Plasmids were injected i.m. and immune response was assessed by T cell and antibody response against CEA and tumor protection.
Results/Discussion: *In vitro* studies validated that the fusion proteins were produced, secreted and recognized by both anti-CEA and anti-GM-CSF antibodies. GM-CSF activity was confirmed with a GM-CSF dependent mouse cell line. Immunization of C57/BL6 mice with DNAs encoding the fusion proteins led to T cell and antibody responses against CEA. These responses were comparable to immunization with plasmid DNA encoding full length CEA only. Tumor challenge with CEA-expressing syngeneic mouse adenocarcinoma cells (MC38-CEA) resulted in development of large tumors in control groups by day 25. In contrast, no tumors were noted in any of the CEA or CEA-GM-CSF immunized groups at this time. Subsequently, tumors developed at approximately day 35 in more than half of those animals immunized with the CEA-GM-CSF fusions, while only 1/20 in the plasmid CEA alone groups developed tumors. Further evaluations demonstrated that mice injected with the CEA-GM-CSF fusion plasmids developed IgG autoantibodies to mGM-CSF, and that these antibodies neutralized mGM-CSF activity *in vitro*. Mice injected with plasmid DNA encoding GM-CSF alone did not produce such antibodies. A single, low dose immunization with fusion plasmids resulted in lower titers of anti-mGM-CSF antibodies better tumor protection than CEA encoding plasmid alone.